Inherited Dystrophic Epidermolysis Bullosa in Inbred Dogs: A Spontaneous Animal Model for Somatic Gene Therapy

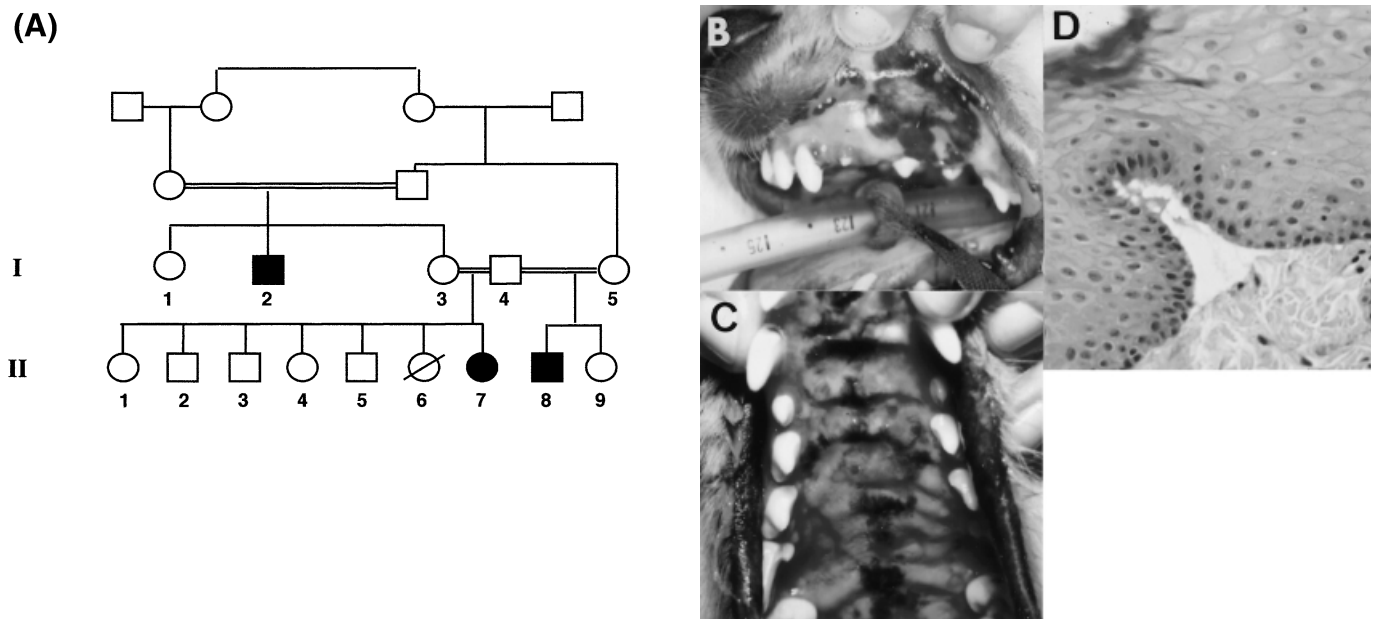

Figure 1. Oral erosions in Golden Retriever dogs suffering from a mild form of dystrophic EB. The dystrophic EB dogs (individuals II-7 and II-8) are products of two distinct inbred unions within a strain of animals with a previous history of inherited skin blistering disease. Dog 1-4 had ancestors common to dogs I-3 and I-5 (*A*). Ulcerations and hemorragic blisters of the oral mucosa and lips in the affected female and the male dog (*B* and *C*, respectively). Histologic examination of the blistered skin detects a subepidermal cleavage (*D*).

To the Editor:

To date, the feasibility of gene therapy strategies for inherited skin diseases has been successfully investigated in recessive conditions, comprising dystrophic epidermolysis bullosa (EB) (Sawamura *et al.*, 1999). Dystrophic EB is a mechanobullous disorder of the skin caused by mutations in the gene (COL7A1) encoding collagen type VII. Altered expression of collagen type VII results in blisters caused by intradermal separation occurring beneath the lamina densa, at the level of the anchoring fibrils. Extracutaneous involvement includes lesions of the oral cavity and the gastrointestinal tract. No conventional therapy is available for this disease, and the animal models so far accessible are inappropriate to accurately evaluate the efficacy of the curative transgenes before clinical trials in humans.

We have recently identified a family of inbred dogs with members suffering from a mild form of recessive dystrophic EB. These dogs offer the possibility of testing therapeutic assays based on gene replacement in immunocompetent hosts. The diseased animals, a male and a female, are products of two distinct inbred unions in a family of Golden Retriever dogs with an history of bullous eruptions inherited in an autosomal recessive mode (**Fig 1*A***). The female dogs was the only affected pup of a litter of seven. At birth, punctiform hemorrhages were noted under the tongue and on the hard palate. The lesions rapidly evolved into large multifocal ulcerations and hemorragic blisters of the whole oral mucosa and lips (**Fig 1*B***). Endoscopy revealed involvement of the esophageal mucosa. The skin presented a generalized tendency to blister. Milium, which is infrequent in canine dermatopathology, and erythema were noted on the ventral abdomen. The active blistering of the skin ceased at the age of 8 months, whereas formation of blisters and erosions of the upper digestive tract persisted. Growth retardation, possibly linked to difficult feeding, developed with aging. The male dog was delivered by cesarean together with another littermate. A labial ulceration formed *in utero* was noted in the diseased pup at the rupture of the amniotic envelope. Shortly after birth, the dog developed skin blisters and erosions of the digestive tract that presented the clinical pattern and evolution previously observed in the affected EB female (**Fig 1*C***).

Biopsies were obtained from involved and non-involved areas of the skin and mucosal epithelia of the two dogs at the age of 8 wk. Histologic examination of 4-μm paraffin-embedded sections of fixed skin stained with hematoxylin and eosin and Schiff periodic-acid detected a subepidermal cleavage in the blistered areas of the integument (**Fig 1*D***). The basement membrane of the dermal–epidermal junction was located at the roof of the blisters. Electronmicroscopic examination confirmed the intradermal localization of the cleavage plane in the blistered skin, and showed presence of ultrastructurally normal hemidesmosomes and anchoring fibrils (not shown). To confirm the diagnosis of dystrophic EB, biopsy specimens obtained from the skin, oral mucosa and esophagus were processed for immunofluorescence examination using antibodies directed against the major components of the basement membrane of the dermal–epidermal junction. Compared with healthy unrelated controls (**Fig 2*A***), reactivity to monoclonal antibody LH7:2, directed against collagen type VII (Heagerty *et al.*, 1986), was slightly reduced in the skin of the female dog and strongly decreased in the male (**Fig 2*B*, *C***). Rare basal keratinocytes displayed a bright fluorescence indicating intracytoplasmic retention of collagen type VII molecules. In both animals, immunoreactivity to collagen type VII of the oral and esophageal epithelia was weak (**Fig 2*E***, ***F*** and ***H***, ***I***, respectively). Labeling of the

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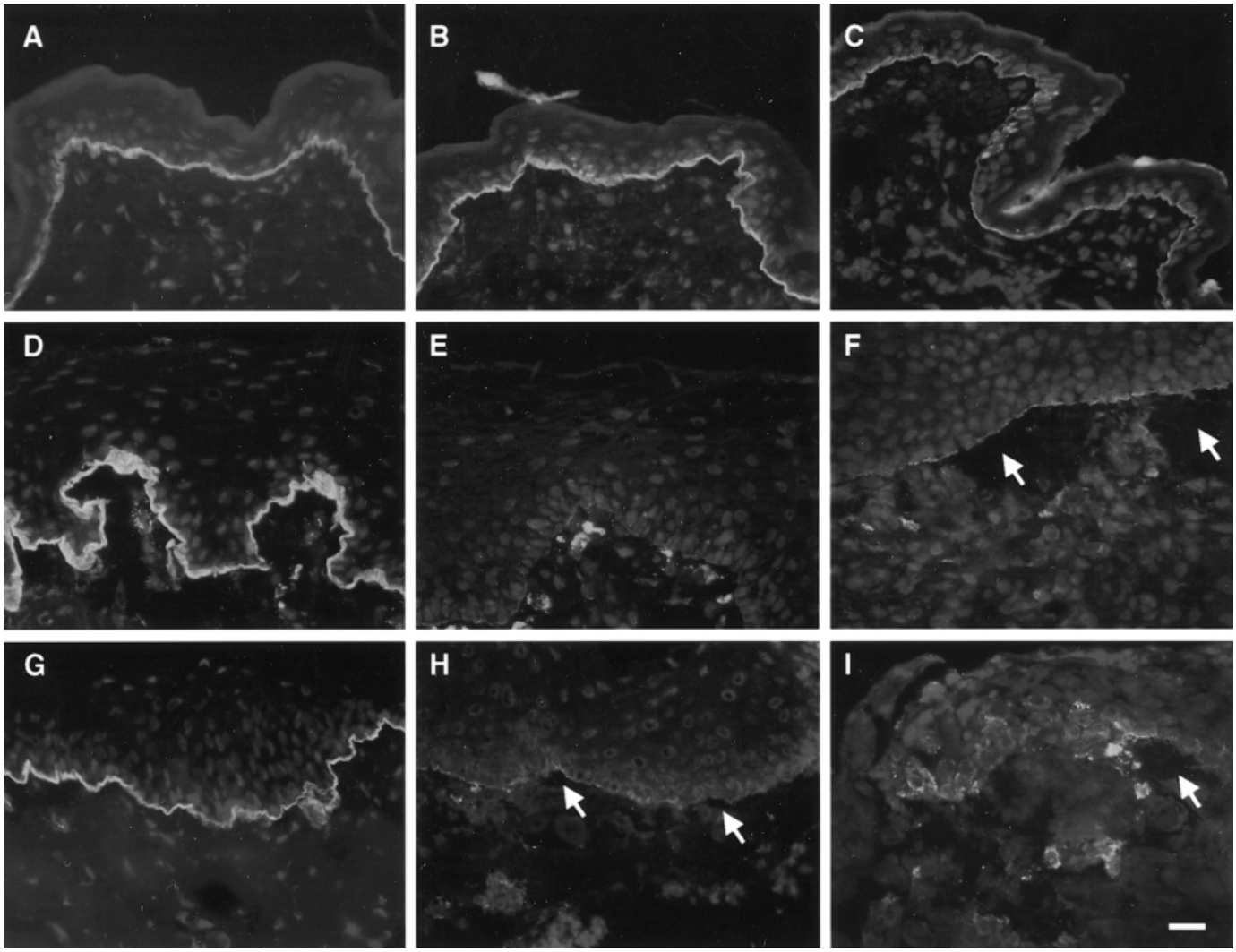

Figure 2. Altered expression of collagen type VII in canine dystrophic EB epithelia. Immunofluorescence analysis of frozen cryostat sections of epithelia specimens obtained from a healthy unrelated control (*A*, *D*, *G*), the dystrophic EB female (*B*, *E*, *H*) and male (*C*, *F*, *I*) dogs were stained with monoclonal antibody LH7:2 specific to collagen type VII. Compared with the control skin (*A*), non-lesional skin of the dystrophic EB female dog revealed a slightly reduced reactivity (*B*) while non-lesional skin of the diseased male was weakly immunoreactive (*C*). Reactivity of the oral and esophageal epithelia (*E*, *F* and *H*, *I*, respectively) appeared strongly reduced in the two affected animals. Labeling of the basement membrane was continuous at the roof of the blisters (*F*, *H*). Basilar, suprabasilar, and intradermal staining of collagen type VII was also observed. Blistered areas are indicated (*arrows*). *Scale Bar*: 20μm.

basement membrane was continuous and located on the roof of the blisters (**Fig 2*F*, *H***). Basilar, suprabasilar, and intradermal staining of collagen type VII was observed at time of active blistering. The clinical, histologic and immunohistologic observations were therefore consistent with the diagnosis of recessive dystrophic EB.

Identification of a family of inbred dogs with dystrophic EB is of relevance, because these animals constitute the unique animal model so far identified suitable for a gene therapy approach of the condition. To establish an animal model of dystrophic EB, transgenic collagen type VII-null mice have recently been constructed that present the hallmarks of severe dystrophic EB (Heinonen *et al*., 1999). These mice, however, die shortly after birth, which renders their use in gene replacement studies problematic. Sporadic cases of hereditary blistering disorders have been reported in cattle, foals, dogs, cats and mouse (Jolly *et al*., 1974; Frame *et al*., 1988; Agerholm, 1994; Kyuster *et al*., 1997). In most cases the diagnosis drawn from clinical observations was not confirmed by immune epitope mapping or electron microscopic examination of the blistered tissues. Severe recessive dystrophic EB associated with absent expression of collagen type VII was described in sheep, but early exungulation and severe oral erosions leading to difficulty in feeding compromise the survival of the affected lambs and consequently their use to devise curative protocols (Bruckner-Tuderman *et al*., 1991). A localized form of DEB, presenting with podal and oral ulcerative lesions with no equivalent in humans, and a generalized DEB form manifesting with cutaneous and mucosal erosions have also been described in two unrelated cats (White *et al*, 1994; Olivry *et al*., 1999). An isolated case of mild dystrophic EB in a Japanese Akita Inu dog has also been reported, in which the condition was characterized by positive immunoreactivity to collagen type VII and reduction in the number of anchoring fibrils (Nagata *et al*., 1995). The family history of all these EB animals was imcomplete, however, and the inheritance mode of the disease unclear.

The intracytoplasmic retention of collagen type VII, and the favorable course of the clinical disease activity observed in the Golden Retriever dogs, also constitute the hallmark of a form of dystrophic EB designated ''transient bullous dermolysis of the newborn'' (TBDN) (Fine *et al*., 1993). TBDN is associated with genetic mutations in the COL7A1 gene causing temporary blistering of the integument (Christiano *et al*., 1997; Hammami-Hauasli *et al*., 1998). In our dogs, the self-limiting course distinctive

of TBDN is not fully observed, because the mechanical fragility of the digestive epithelia persists in adulthood. Interestingly, blistering localized to the upper digestive tract in the absence of cutaneous involvement has recently been observed in humans, in a family presenting with a specific form of inherited dystrophic EB (L. Bruckner-Tuderman and W. Kuester, personal communication).

In conclusion, these Golden Retriever dogs provide an animal model that contributes to the study of the pathomechanisms associated with an aberrant expression of collagen type VII. They also provide the unique means so far available to test the *in vivo* delivery of therapeutic transgenes in immunocompetent animals. In this respect, gene targeting to oral and digestive mucosa in the canine system faithfully reflects the human context because no considerable difference exists between soft epithelia in these anomal models and the human counterparts.

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